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Single Vaccination Provides Limited Protection to Ducks and Geese Against H5N1 High Pathogenicity Avian Influenza Virus

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SUMMARY. Since 2002, high pathogenicity avian influenza (HPAI) has spread from Asia to Europe and into Africa, causing the largest epizootic of HPAI of the last 50 yr, including infecting domestic and wild waterfowl. Our study was conducted to investigate whether a single vaccination of 7-day-old domestic ducks and geese with inactivated oil emulsion vaccines resulted in protection against HPAI virus challenge at 30 days of age. In ducks, some but not all vaccines decreased oropharyngeal and cloacal viral shedding for different periods postchallenge when compared with the sham group. In geese, decreased morbidity signs and mortality were noted but limited to some vaccines. Best protection was seen with a vaccine homologous to HPAI challenge virus. Limited decreases in oropharyngeal and cloacal viral shedding and mixed results were attained when looking at seroconversion. Our results indicate a single dose of oil-emulsified vaccine optimized for chickens did not provide adequate protection for ducks and geese against HPAI virus, and, at a minimum, additional research is needed to formulate waterfowl-specific vaccines.

RESUMEN. La vacunación con una sola aplicación confiere una protección limitada en patos y gansos contra el virus de la influenza aviar de alta patogenicidad H5N1.

Desde el año 2002, la influenza aviar de alta patogenicidad se ha propagado desde Asia hasta Europa y África, causando la mayor epizootia de esta enfermedad en los últimos 50 años, incluyendo la infección de aves acuáticas domésticas y silvestres. Este estudio fue diseñado para determinar si la vacunación con una sola aplicación en patos y gansos domésticos a los siete días de edad utilizando vacunas inactivadas emulsionadas en aceite confieren protección contra el desafío a los 30 días de edad con un virus de la influenza aviar de alta patogenicidad. En los patos, algunas pero no todas las vacunas disminuyeron la eliminación viral por la vía orofaríngea y cloacal por diferentes periodos posteriores al desafío en comparación con el grupo control no vacunado. En los gansos, se observó disminución de la morbilidad, en la mortalidad y en los signos clínicos pero esto se limitó a algunas vacunas. La mejor protección se observó utilizando una vacuna homóloga contra el virus de influenza aviar altamente patógena utilizado en el desafío. Se observaron disminuciones limitadas en la eliminación viral por las vías orofaríngea y cloacal además de que se obtuvieron resultados mixtos en la seroconversión. Estos resultados indican que una sola dosis de vacuna emulsionada en aceite optimizada para pollos no proporciona una protección adecuada contra el virus de la influenza aviar altamente patógeno en patos y gansos y se necesita investigación adicional para formular vacunas específicas para aves acuáticas.

Key words: ducks, geese, H5N1, highly pathogenic avian influenza, vaccine, inactivated vaccine

Abbreviations: AGID = agar gel immunodiffusion; AI = avian influenza; dpc = days postchallenge; EID_{50} = mean embryo infective doses; ENG = A/turkey/Eng/N28/73 (H5N2); GMT = geometric mean titer; HA = hemagglutinin; HGO = A/chicken/HGO/28159-232/95 (H5N2); HI = hemagglutinin inhibition; HPAI = high pathogenicity avian influenza; INDO = A/chicken/Indo/7/03 (H5N1); rFPV-AIV-H5 = recombinant fowl poxvirus with hemagglutinin gene insert from A/turkey/Ireland/84 (H5H9); TK/WI = A/turkey/WI/68 (H5N9); USDA = U.S. Department of Agriculture

In 1996, H5N1 high pathogenicity avian influenza (HPAI) emerged in China and was reported as causing 40% mortality in domestic geese. The disease has spread to cause the largest epizootic of HPAI of the last 50 yr, infecting poultry, various wild birds, some mammals that consumed infected birds, and some lethal and nonlethal cases in humans (1,28). Before 2001, H5N1 HPAI virus was identified mainly in gallinaceous poultry, and infections and mortality in wild or domestic waterfowl were uncommon. Historically, other HPAI viruses have either not been infectious to domestic waterfowl or had limited replication when examined in experimental models. The initial H5N1 HPAI viruses of Guangdong lineage produced limited replication in the respiratory tract of domestic ducks and no mortality (21). In 2001, an H5N1 HPAI virus was isolated from duck meat imported into South Korea and, experimentally, intranasal inoculation of domestic ducks produced asymptomatic infections with virus in multiple organs, including respiratory tissues and meat (35). By the end of 2002, an H5N1 HPAI virus lineage had emerged that infected and killed a wide range of captive waterfowl, including various duck species, in two of Hong Kong's wild bird parks (9,26). Since 2002, HPAI (H5N1) has spread from Asia to Europe and into Africa. The primary reservoir of H5N1 in Southeast Asia has become the domestic duck, both free-range and backyard, where the H5N1 HPAI viruses can cause mortality (10,41). These H5N1 viruses continue to circulate in poultry despite efforts by public health and veterinary authorities to contain the virus (6,7,12,13,14,38,42).

Traditional methods, such as stamping out are no longer a viable option in countries where HPAI has become endemic, especially where domestic waterfowl have become the reservoir of H5N1 HPAI (15,39). In some countries, vaccination has become an option to maintain rural livelihood and food security. However, available vaccines and vaccination protocols have been developed and tested in chickens or other gallinaceous poultry, and limited testing has been conducted on ducks or geese. Vaccine efficacy can be quite different between avian species. Various studies have been done with the use of whole, inactivated vaccines in ducks with varying success (3,4,19,34,36). One study indicated that a two-dose vaccination regimen given in the first 30 days with an inactivated conventional vaccine in Pekin ducks provided protection (3). However, in much

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Table 1. Virus isolation by days postchallenge (dpc)[A] for duck experiment.

Vaccine group	Swabs	Virus isolation (log EID_{50}/ml)[BC] 1 dpc	2 dpc	3 dpc	4 dpc	7 dpc	10 dpc	14 dpc
INDO	Oropharyngeal	4/6 (1.9[b])	3/6 (1.0[b])	1/6[a] (1.0[b])	0/6[a] (0.9[b])	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
	Cloacal	0/6 (0.9)	0/6[a] (0.9)	0/6[a] (0.9)	0/6[a] (0.9)	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
TK/WI	Oropharyngeal	3/6 (1.3[b])	3/6 (1.4[b])	3/6 (1.4[b])	3/6 (1.2[b])	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
	Cloacal	0/6 (0.9)	0/6[a] (0.9)	2/6 (1.0)	1/6 (1.1)	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
HGO	Oropharyngeal	6/6 (2.4)	5/6 (1.7)	4/6 (1.7[b])	4/6 (1.3[b])	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
	Cloacal	0/6 (0.9)	0/6[a] (0.9)	0/6[a] (0.9)	2/6 (0.9)	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
ENG	Oropharyngeal	6/6 (2.5)	5/6 (2.2)	5/6 (2.4)	6/6 (2.0)	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
	Cloacal	0/6 (0.9)	0/6[a] (0.9)	1/6[a] (1.3)	2/6 (1.2)	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
Sham	Oropharyngeal	11/12 (2.0)	11/12 (2.7)	11/12 (3.4)	11/12 (2.3)	2/12 (0.9)	0/6 (0.9)	0/6 (0.9)
	Cloacal	1/12 (0.9)	6/12 (1.5)	8/12 (1.7)	7/12 (1.3)	0/12 (0.9)	0/12 (0.9)	0/12 (0.9)

[A]Number of positives/total numbers tested; lowercase letter indicates significant difference ($P < 0.05$) between individual vaccine group and sham group.
[B]Virus shed titer (log titer/ml); lowercase letter indicates significant differences ($P < 0.05$) between individual vaccine groups and sham group for titer.
[C]The minimal detection limit used (mean embryo infective doses, EID_{50}) was 0.9 log EID_{50}/ml.

of Southeast Asia, domestic duck production focuses on placing ducklings in the rice fields beginning at 3–4 wk of age, suggesting the need for competent protection at this time because of potential exposure to infected wild waterfowl and other domestic ducks in the field (3,24).

This study was conducted to investigate whether a single vaccination of 7-day-old Pekin ducks and Chinese geese with inactivated oil emulsion vaccines containing H5 seed strains could protect against challenge from HPAI virus strain A/chicken/Indonesia/7/03 (H5N1) at 30 days of age.

MATERIALS AND METHODS

Challenge virus. Nine-day-old embryonating chicken eggs were used to grow challenge virus stocks of A/chicken/Indonesia/7/03 (H5N1). This virus was isolated from a diagnostic specimen submitted from a farm experiencing high mortality in broiler chickens in Indonesia in early December 2003 during an HPAI outbreak. This virus resulted in high mortality and high quantities of virus shed from respiratory and intestinal tracts of intranasally inoculated chickens in experimental studies. We chose this virus because it is a clade 2.1 H5N1 HPAI virus from an HPAI outbreak in Indonesia. Viruses were passaged twice, and allantoic fluid was collected. Brain-heart infusion medium was used to dilute allantoic fluid to a final titer of 10^6 mean embryo infective doses (EID_{50}) per 0.1 ml, as previously described.

Animals and housing. Animals were cared for and housed in compliance with an Institutional Animal Care and Use Committee approved animal use protocol at the Southeast Poultry Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture (USDA), Athens, GA. All experiments were performed in a USDA-certified Biosafety Level 3-Enhanced facility.

Vaccines. Vaccine viruses were grown in 10-day-old specific pathogen-free embryonating chicken eggs and the infective chorioallantoic fluid was pooled for each avian influenza (AI) virus isolate in each experiment. Viruses used were A/turkey/WI/68 (H5N9) (TK/WI), A/chicken/Hidalgo/28159-232/95 (H5N2) (HGO), A/turkey/England/N28/73 (H5N2) (ENG), and A/chicken/Indonesia/7/03 (H5N1) (INDO). Vaccine was made as previously described (31). Hemagglutination titers and infectious titers were determined before inactivation. Hemagglutination titers were 128 (INDO and ENG) and 512 (TKWI and HGO), and infectious titers were $10^{8.7}$ (INDO), $10^{9.3}$ (TKWI), $10^{8.9}$ (HGO), and $10^{8.3}$ (ENG) EID_{50}/ml. Inactivation was confirmed by chicken embryo inoculation (24). A commercial vaccine containing a recombinant fowl poxvirus genetically engineered to contain the hemagglutinin (HA) gene insert from A/turkey/Ireland/84 (H5N9) (rFPV-AI-H5) was used for Experiment 2 (geese only). Geese were inoculated at 7 days of age, subcutaneously in the nape of the neck with 0.2 ml per bird of rFPV-AI-H5 vaccine as per the manufacturer's instructions. For all inactivated vaccines (TK/WI, HGO, ENG, INDO, and sham), ducks and geese were inoculated at 7 days of age subcutaneously in the nape of the neck with 0.5 ml of vaccine. Shams received an inoculation of oil-emulsified sterile allantoic fluid with the same vaccine protocol as above (31).

Experimental design. Seven-day-old white Pekin ducks (McMurray Hatcheries, Webster City, IA) and white Chinese geese (Privett Hatchery, Portales, NM) were vaccinated subcutaneously in the nape of the neck. Three weeks postvaccination, ducks and geese were challenged intranasally with high pathogenicity A/chicken/Indonesia/7/03 (H5N1) diluted to contain 10^6 EID_{50} per 200-μl dose. Individual oropharyngeal and cloacal swabs were taken on 0, 1, 2, 3, 4, 7, 10, and 14 days postchallenge (dpc) and individually stored frozen at −70 C until tested. Blood was taken for serum before vaccination, 3 wk postvaccination and 2 wk postchallenge. Ducks and geese were euthanatized at 2 wk postchallenge.

Serology. Serum was assayed for AI virus–specific antibodies by hemagglutinin inhibition (HI) and agar gel immunodiffusion (AGID) assays, as previously described (2,30). Serologic results from the HI test are presented as geometric mean titers (GMTs). Test antigen from the National Veterinary Services Laboratory (Ames, IA) was used to detect precipitating antibodies for the AGID assay.

Virus isolation. Virus isolation was performed as previously described with the use of 9- to 11-day-old embryonating chicken eggs (30,31). EID_{50} was determined by further titration of positive samples in 9- to 11-day-old embryonating chicken eggs. The minimal detectable titer from the swabs was $10^{0.91}$ EID_{50}/ml.

Statistical analysis. Statistical analysis was performed by SAS version 9.1. ANOVA was carried out, and Duncan's New Multiple Range Test was used to analyze the repeated measures data for both ducks and geese and for both oropharyngeal and cloacal viral shed for days postinfection. For analysis of viral shed, 0.91 log was used as the minimal level of detection that was considered positive. Alpha was 0.05.

RESULTS

Experiment 1 (ducks). No morbidity or mortality was observed in the vaccine or sham groups after challenge with H5N1 HPAI virus.

Oropharyngeal and cloacal shedding. The number of ducks shedding challenge virus and the amount of viral shedding was determined for both oropharyngeal and cloacal samples at 1, 2, 3, 4, 7, 10, and 14 dpc The TK/WI, HGO, and ENG vaccines did not result in significant reductions in the number of ducks from which virus could be isolated in oropharyngeal samples for any of the days, compared with the dpc-matched sham group (Table 1). Vaccine INDO had significant reduction in numbers of ducks that virus

Table 2. Indirect assessment of vaccine (vacc.) protection[A] by measuring serologic response in ducks.[B]

Vaccine group	HI antigen source	HI (GMT)			AGID		
		At vacc.	3 wk postvacc.	2 wk postchallenge	At vacc.	3 wk postvacc.	2 wk postchallenge
INDO	Vaccine strain	0/6 (<8)	6/6 (12)	6/6 (91)	0/6	6/6	6/6
	Challenge strain	0/6 (<8)	6/6 (12)	6/6 (91)[a]			
TK/WI	Vaccine strain	0/6 (<8)	6/6 (13)	6/6 (91)	0/6	6/6	6/6
	Challenge strain	0/6 (<8)	0/6 (<8)	6/6 (39)[a]			
HGO	Vaccine strain	0/6 (<8)	6/6 (23)	6/6 (147)	0/6	6/6	6/6
	Challenge strain	0/6 (<8)	0/6 (<8)	6/6 (104)[a]			
ENG	Vaccine strain	0/6 (<8)	6/6 (16)	6/6 (446)	0/6	6/6	6/6
	Challenge strain	0/6 (<8)	0/6 (<8)	6/6 (111)			
Sham	Vaccine strain	NA	NA	NA	0/12	0/12	12/12
	Challenge strain	0/12 (<8)	0/12 (<8)	12/12 (223)			

[A]Number positive/total (GMT), lowercase letter indicates significant difference ($P < 0.05$) between individual vaccine group and sham group.
[B]AGID = agar gel immune-diffusion assay; GMT = geometric mean titer; HI = hemagglutinin inhibition assay; NA = not applicable.

could be isolated from oropharyngeal samples 3 and 4 dpc compared with sham group.

Significant differences in the titers of virus shed were found for repeated measures between groups ($P < 0.0001$), as well as significant differences when comparing vaccine groups on certain days ($P < 0.0001$). When compared with the sham groups, significantly reduced oropharyngeal titers were detected 1 dpc (INDO and TK/WI group), 2 dpc (INDO and TW68), and 3 and 4 dpc (INDO, TK/WI, and HGO) (Table 1). Analysis for 7, 10, and 14 dpc for duck oropharyngeal shedding yielded no differences when compared with the sham groups for the respective days.

The vaccines reduced the number of ducks shedding virus in cloacal swabs taken, compared with the sham group (Table 1). A significant reduction in the number of ducks shedding challenge virus cloacally was noted 2 dpc for INDO, TK/WI, HGO, and ENG vaccine groups; 3 dpc for INDO, HGO, and ENG vaccine groups; and 4 dpc for INDO vaccine. However, no significant differences in the virus titer of cloacal swabs were seen between any vaccine groups and the sham group (Table 1).

Serology. All groups were seronegative by HI test at the time of vaccination. Antibody response to the respective vaccine virus was seen by 3 wk postvaccination, and response to the challenge virus strain was seen 2 wk postchallenge in every group. With the use of vaccine strain as antigen, the GMTs at 3 wk postvaccination for INDO, TK/WI, HGO, and ENG vaccine groups were 12, 13, 23, and 16 , respectively, and 91, 91, 147, and 446, respectively, 2 wk postchallenge (Table 2). The sham group HI titers were <8 GMT postvaccination. With the challenge strain as antigen, the HI titers postchallenge were 91, 39, 104, 111, and 223 for INDO, TK/WI, HGO, ENG, and sham groups, respectively (Table 2). Significantly lower antibody titers were seen for the INDO, TK/WI, and HGO groups compared with the sham group when comparing the postchallenge response using the challenge strain (Table 2).

All groups were seronegative by AGID assay at time of vaccination. At 3 wk postvaccination, all vaccine groups had seroconverted, except the sham group, as evidenced by the positive AGID tests. At 2 wk postchallenge, all ducks in all groups were seropositive.

Experiment 2 (geese). *Morbidity and Mortality*. After challenge, signs of morbidity, such as splayed legs, inability to walk without staggering, torticollis, dysmetria, listlessness, and general unresponsiveness, were seen in the geese groups with various levels of mortality. Nine of 12 geese in the sham group died or were euthanatized for humane reasons, with a mean death time of 5.1 days. Two geese were euthanatized for humane reasons in the ENG group, with a mean death time of 5.5 days. On 7 dpc, the sham group only had three surviving geese, which survived through 14 dpc. The INDO, TK/WI, HGO, and rFPV-AIV-H5 groups had all geese in each group survive until the end of the study. The ENG group had four geese that survived from 7 to 14 dpc. Statistically significant differences ($P < 0.05$) were seen for mortality in the geese study for all vaccine groups when compared with the sham group.

Oropharyngeal and cloacal shedding. Viral titers from oropharyngeal and cloacal swab samples taken at 1, 2, 3, 4, 7, 10, and 14 dpc were analyzed. Statistically significant differences were found for repeated measures between groups ($P < 0.0001$), as well as when comparing vaccine groups on certain days ($P < 0.0001$).

A significant reduction in the number of geese with virus reisolated from oropharyngeal swab samples on day 3 was seen for the INDO vaccine group, but not for TK/WI, HGO, ENG, HGO, or rFPV-AIV-H5 vaccine groups compared with the sham group (Table 3). Significant reductions in oropharyngeal titers shed were detected on 2 dpc (INDO, TK/WI, and HGO groups), 3 dpc (INDO and HGO groups), and 4 dpc (INDO, TK/WI, and HGO groups) when compared with the sham groups (Table 3).

Significant differences for repeated measures were found between groups ($P < 0.01$), as well as between vaccine groups on certain days ($P < 0.0001$) for cloacal shedding. Cloacal shedding was seen in all groups of the goose study, with the highest levels observed on 3 and 4 dpc (Table 3). The number of animals with measurable virus levels from cloacal swab samples for each group for each time point was also recorded. A statistically significant reduction in the number of geese from which virus could be isolated in cloacal samples was seen only on day 4 for the HGO vaccine group compared with the sham group. Significantly higher differences in titer levels were found on 4 dpc for the ENG and rFPV-AIV-H5 vaccine groups compared with the sham group.

Serology. None of the vaccine groups, including the sham group, had an HI or AGID antibody response at time of vaccination (Table 4). On the basis of AGID 3 wk postvaccination, partial seroconversion was observed for the INDO group (2/6 geese) and HGO group (4/6 geese), full seroconversion was observed for the TK/WI (6/6 geese) group, and no seroconversion was observed for the ENG (0/6 geese) or rFPV-AIV-H5 (0/6 geese) groups. At 2 wk postchallenge, all surviving geese in all groups were seropositive.

With the vaccine strain as antigen, HI titers were low for all groups 3 wk postvaccination; that is, INDO, TK/WI, HGO, ENG, and rFPV-AIV-H5 vaccine groups had GMT of 23, 37, 26, 5, and 8, respectively. At 2 wk postchallenge, the HI titers using vaccine strain as antigen were higher but variable, being 223, 2896, 512, 45, and 56 GMT for INDO, TK/WI, HGO, ENG, and rFPV-AIV-H5, respectively. With the challenge virus as antigen, postchallenge HI

Table 3. Virus isolation by days postchallenge (dpc)[A] for goose experiment.

Vaccine group	Swabs	Virus isolation (log EID_{50}/ml)[BC] 1 dpc	2 dpc	3 dpc	4 dpc	7 dpc	10 dpc	14 dpc
INDO	Oropharyngeal	5/6 (2.7[b])	4/4 (2.5[b])	4/6[a] (2.3[b])	4/6 (2.1[b])	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
	Cloacal	0/6 (0.9)	6/6 (1.2)	6/6 (1.7)	6/6 (1.6)	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
TK/WI	Oropharyngeal	4/6 (2.7[b])	6/6 (3.6[b])	6/6 (3.8)	6/6 (3.6[b])	1/6 (1.0)	0/6 (0.9)	0/6 (0.9)
	Cloacal	3/6 (1.0)	6/6 (1.6)	6/6 (3.1)	6/6 (2.1)	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
HGO	Oropharyngeal	6/6 (3.8)	4/6 (2.0[b])	6/6 (3.0[b])	5/6 (2.7[b])	1/6 (1.2)	0/6 (0.9)	0/6 (0.9)
	Cloacal	0/6 (0.9)	4/6 (1.9)	5/6 (3.0)	4/6[a] (3.2)	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
ENG	Oropharyngeal	6/6 (3.5)	6/6 (4.2)	6/6 (4.4)	6/6 (4.8)	0/4 (0.9)	0/4 (0.9)	0/4 (0.9)
	Cloacal	1/6 (1.0)	6/6 (2.4)	5/6 (3.5)	6/6 (4.2[b])	0/4 (0.9)	0/4 (0.9)	0/4 (0.9)
rFPV-AIV-H5	Oropharyngeal	6/6 (3.2)	6/6 (3.5)	6/6 (4.1)	6/6 (4.3)	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
	Cloacal	2/6 (0.9)	5/6 (2.0)	6/6 (3.0)	6/6 (4.0[b])	0/6 (0.9)	0/6 (0.9)	0/6 (0.9)
Sham	Oropharyngeal	11/12 (4.0)	12/12 (4.7)	12/12 (4.5)	12/12 (5.0)	1/3 (1.3)	0/3	0/3
	Cloacal	4/12 (0.9)	12/12 (2.0)	12/12 (2.8)	12/12 (3.0)	0/3	0/3	0/3

[A]Number of positives/total numbers tested; lowercase letter indicates significant difference ($P < 0.05$) between individual vaccine group and sham group.

[B]Virus shed titer (log titer/ml); lowercase letter indicates significant differences ($P < 0.05$) between individual vaccine groups and sham group for titer.

[C]The minimal detection limit (mean embryo infective doses, EID_{50}) used was 0.9 log EID_{50}/ml.

GMT titers were 223, 512, 256, 181, 194, and 256 for INDO, TK/WI, HGO, ENG, rFPV-AIV-H5, and sham groups, respectively (Table 4).

DISCUSSION

In this study, 1-wk-old conventional domestic ducks and geese were vaccinated once and challenged 3 wk later with A/chicken/Indonesia/7/03 (H5N1) HPAI virus. This study demonstrated species differences between ducks and geese in vaccine efficacy parameters that can be evaluated to determine protection, including mortality rates, numbers of animals shedding challenge virus, the quantity of challenge virus shed orally and cloacally, serologic titers elicited, and numbers of animals that seroconvert. In the sham duck group, there was no mortality, but in the sham goose group, mortality was high, making mortality a measurable metric for protection. When looking at mortality in the goose study, all vaccines provided protection compared with the sham group. In measuring protection in ducks, statistical differences were noted for decreased quantity of oral virus shedding for vaccine groups INDO (days 1, 2, 3, and 4), TK/WI (days 1, 2, 3, and 4), and HGO (days 3 and 4) when compared with the sham group. Similarly, in geese, statistical differences were noted for decreased virus shed orally for vaccine groups INDO (days 1, 2, 3, and 4), TK/WI (days 1, 2, and 4), and HGO (days 2, 3, and 4) and for cloacal virus shed in vaccine group ENG (day 4) and rFPV-AIV-H5 (day 4) when compared with the sham group. In general, the vaccine made from the challenge virus (INDO) was most consistent in providing protection in both ducks and geese in terms of preventing mortality and in limiting the quantity and time window of virus shedding. Serologically, all ducks vaccinated with the INDO vaccine had AGID antibodies by 3 wk postvaccination, whereas only two of six geese had HI antibodies by 3 wk postvaccination. In ducks, at the time of influenza infection, the vaccine groups INDO, TK/WI, HGO, and ENG had an HI response using the vaccine strain as antigen. Two weeks postinfection, all groups had a response against the challenge virus, with a higher HI titer when using the vaccine strain as antigen (Table 2). In geese, at the time of infection, vaccine groups INDO, TK/WI, HGO, ENG, and rFPV-AIV-H5 had an HI antibody response against the vaccine strain. At 2 wk postinfection, all vaccine groups showed an HI antibody response against the challenge strain (Table 4). The AGID was also completed, but gave inconsistent positive results, as has been previously reported for influenza A virus in infected domestic waterfowl. In this study, we noted a lack of seroconversion in the geese, but all ducks seroconverted when using AGID. By contrast, single vaccination of chickens with similar inactivated oil emulsion H5 vaccines provided consistent prevention of morbidity, mortality, and high HI serum titers; consistent AGID antibody response; and reduced virus replication and shedding after H5N1 HPAI virus challenge (5,11,29,32).

Few studies have been undertaken to ascertain the effect of vaccines on ducks, and even fewer studies on geese, despite ducks and geese being a valuable and sustainable food source in Southeast Asia, Africa, and parts of Europe. One study investigated the protection elicited with the use of different concentrations of HA protein in a single immunization against a highly lethal H5N1 HPAI virus and found 1 μg of HA protein was sufficient to provide protection (17). However, the study also stated that the HA protein concentration needed in a vaccine might be different for commercial vaccines that are not purified and concentrated (17). A study conducted with ducks using A/Chicken/China/1204/04 as the challenge virus and A/Chicken/Mexico/232/94/CPA (H5N2) as the vaccine seed strain significantly reduced excretion and transmission of H5N1 HPAI with single vaccination (37). Steensels *et al.* (25) showed that a prime-boost strategy stimulated broader immunity in ducks. In another study, geese and ducks were vaccinated with a high-growth, low pathogenicity H5N1-inactivated vaccine developed by reverse genetics. The HA and the NA genes were from A/goose/Guangdong/1/96, and the six internal genes were from A/Puerto Rico/8/34 (PR8). Ducks showed no mortality and reduced shedding after vaccination and administration of a booster vaccination, whereas geese showed decreased mortality and reduced shedding when challenged, but only after administration of two booster vaccinations (34). Rudolf *et al.* (23) conducted a study showing geese vaccinated multiple times were protected from disease but could still be infected and shed virus, although this infection and shed period was shorter than in unvaccinated controls. Pekin ducks in another study were vaccinated twice and proved to be clinically resistant to virus infection and disease with very minimal shedding. Pekin ducks in another study were vaccinated once and then challenged 2 wk postvaccination, and although there was protection from disease, viral shedding was still observed (22). In our study, we

Table 4. Indirect assessment of vaccine (vacc.) protection[A] by measuring serologic response in geese.[B]

		HI (GMT)			AGID		
Vaccine group	HI antigen source	At vacc.	3 wk postvacc.	2 wk postchallenge	At vacc.	3 wk postvacc.	2 wk postchallenge
INDO	Vaccine strain	0/6 (<8)	6/6 (23)	6/6 (223)	0/6	2/6	6/6
	Challenge strain	0/6 (<8)	6/6 (23)	6/6 (223)			
TK/WI	Vaccine strain	0/6 (<8)	6/6 (37)	6/6 (2896)	0/6	6/6	6/6
	Challenge strain	0/6 (<8)	0/6 (<8)	6/6 (512)			
HGO	Vaccine strain	0/6 (<8)	5/6 (26)	6/6 (512)	0/6	4/6	6/6
	Challenge strain	0/6 (<8)	0/6 (<8)	0/6 (256)			
ENG	Vaccine strain	0/6 (<8)	6/6 (5)	4/4 (45)	0/6	0/6	4/4
	Challenge strain	0/6 (<8)	0/6 (<8)	4/4 (181)			
rFPV-AIV-H5	Vaccine strain	0/6 (<8)	2/6 (8)	5/5 (56)	0/6	0/6	5/5
	Challenge strain	0/6 (<8)	0/6 (<8)	5/5 (194)			
Sham	Vaccine strain	NA	NA	NA	0/6	0/6	1/1
	Challenge strain	0/12	0/12	3/3 (128)	0/12	0/12	1/12

[A]Number positive/total.
[B]AGID = agar gel immune-diffusion assay; GMT = geometric mean titer; HI = hemagglutinin inhibition assay.

observed vaccinated geese to have no or decreased mortality, but on some days, the amount of shedding was comparable with the shedding observed from the shams. Challenge virus shedding was also observed for ducks. This observation for both ducks and geese leads to the question of what protection is and how best to define it when looked at the big picture, which includes level of environmental contamination.

The inconsistency of clinical signs in ducks with various HPAI viruses, dynamic host range, and an evolving, enzootic situation coupled with intercontinental spread make finding a suitable vaccine for domestic waterfowl very important (18,20,40). Differences in efficacy in waterfowl species could be related to the level of antigenic homology between challenge and vaccine strains, quantity of antigen in the vaccine, adjuvant that is not optimized for ducks, geese, or both, or differences in vaccine-induced immune responses of ducks and geese compared with chickens. Additional research is needed to optimize inactivated AI vaccines for domestic ducks and geese.

Ideally, a low pathogenicity vaccine seed strain that is antigenically matched to the circulating virus, induces cross-protection against viruses from the same hemagglutinin subtype, and will grow well in eggs would be suitable for vaccine development (8,16). Most of the H5N1 viruses from Asia are of high pathogenicity and therefore not ideal candidates for vaccine seed strain selection (34) because of the need for high-level biocontainment production facilities and the difficulty in attaining suitable levels of virus in embryonating chicken eggs (27,33,43). On the basis of our studies, one dose of oil-emulsified vaccine produced for chickens might be inadequate in ducks and geese to provide optimal protection, even when antigenically closely matched to the challenge virus. Inappropriate vaccine regimens or use of low-potency vaccines leading to incomplete protection, as evidenced by shedding, could allow for transmission and contribute to continuing circulation and spread of H5N1 HPAI viruses. Because of the differences in serologic and shedding responses between ducks and geese, more research is needed to discern and test suitable vaccine candidates and to develop appropriate vaccines and vaccination regimens for ducks and geese given the species differences from gallinaceous poultry. Additional research is needed on improving adjuvants for duck and goose vaccines to improve serologic responses and protection with fewer doses of vaccine.

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